1994 STANFORD NASA-ASEE SUMMER PROGRAM
First Year Report

Orientation Response of Mammalian Cells to Mechanical Force

Andrew O. Martinez
Associate Professor, Division of Life Sciences
University of Texas at San Antonio
San Antonio, Texas

Introduction

My research project was conducted in the Life Sciences Division at the NASA-AMES Research Center in Moffet Field, California. The research was overseen by my colleague, Dr. Rose Grymes. Investigations in Dr. Grymes' laboratory focus on the effects of mechanical forces applied to cultured human cells.

Cell and tissues in the human body are subjected to both passive and active forces originating either from tension created by the cells themselves, or from the environment. For example: (1) Endothelial cells lining the arteries and veins are subjected to pulsations of blood as the heart pumps blood; (2) Underlying smooth muscle cells are subjected to strain due to stretching of the compliant aortic wall; (3) Bone cells are subjected to various degrees of mechanical strain and/or compression, depending on the activity level of the individual; and (4) Skin cells are subjected to biaxial tension during growth, especially in areas of hyperelasticity such as the axial regions, joints and neck.

Standard cell culture techniques generally ignore the importance of mechanical activity to cells. Growth of cells *in vitro* usually occurs on hard-bottomed culture vessels in a static environment. For example, myoblasts cultured *in vitro* fuse to form myotubes that lie in random arrays. However, the application of mechanical stretch induces *in vitro* myoblast cultures to produce myotubes with an aligned morphology very similar to that observed *in vivo* [1]. Sensitivity to applied mechanical force has been investigated using many differentiated cell types: vascular endothelial cells, osteoblasts, fibroblasts, myoblasts, and lung epithelial cells. Effects on mitogenesis, differentiation, gene expression, protein synthesis and cell morphology have been demonstrated [2-7]. This report describes the effect of mechanical force on the orientation (alignment) behavior of normal, transformed, cancer and mutant human and rodent cell lines.

Methods and Materials

Human normal lung fibroblasts (WI38), and SV40 virally-transformed lung fibroblasts (WI38SV40) were obtained from the Coriell Cell Repository (Camden, NJ). Hamster (B14) and mouse (L-cells) tumor and cadherin-deficient mutant cell lines were obtained from my laboratory at the University of Texas at San Antonio. The various cell lines were grown in Dulbecco's modified Eagle's medium (DMEM) containing 50 U/ml penicillin, 50 μg/ml streptomycin and 2 mM L-glutamine. The medium was supplemented with 10% fetal bovine serum. Cells were routinely dissociated from the culture substratum at 80% confluence using 0.05% trypsin/0.53 mM EDTA (ethylene diaminotetra-acetic acid), collected by centrifugation and resuspended in fresh complete DMEM for continued cultivation at a density of 10^4 cells/ml.

Two types of six-well plates (24 mm diameter/well) were obtained from Flexcell International Corp. (McKeesport, PA). On the Flex I plates, the culture well bottoms consist of flexible silicon elastomer membranes. Flex II plates are controls in which the silicon elastomer membranes are supported by a rigid polystyrene molded bottom. The Flex II plate well bottoms are pliable, but cannot be subjected to mechanical stretch. In both types of Flex plates, the silicon elastomer is approximately 3 mm thick and the substrate material is available from the manufacturer with coatings that range from simple A (free amino (NH2) groups) to more complex (collogen type I fragments). All cell lines were adapted to growth on the pliable silicon elastomer membrane coated with collogen type I prior to exposure to mechanical force.

An instrument, Flexercell Strain Unit (Flexcell International Corp, Mckeesport, PA), capable of simulating *in vivo* conditions of mechanical activity in a reproducible, regulated *in vitro* environment was used to apply mechanical force (stretch) to the cells. This unit is designed to apply reproducible cyclic or static tension or compression to cells cultured *in vitro*. It uses air pressure to positively or negatively deform the flexible silicon elastomer membranes that comprise the culture surfaces of the Flex I plates. Moreover, the researcher can select deformation parameters *in vitro* that match those of the cell/tissue *in vivo*.

For the study reported here, 10^4 cells were seeded per well of Flex I and II plates and allowed to attach overnight in DMEM medium. The following morning Flex I plates were seated in the Flexercell Strain Unit baseplate and the cells exposed to a stretch (20% elongation)/relaxation regimen of 6.67 cycles/min (6 seconds of stretch followed by 3 seconds of relaxation) at approximately -12 kPascals for five days. Photomicrography was performed at 24 hr intervals with a Nikon inverted Diphot TMD microscope and attached Nikon N6006 35 mm camera.

Results and Discussion

The Flexercell Strain Unit generates a uniaxial force (stretch) gradient on cells cultured on flexible silicon elastomer membranes (Figure 1). Visual observation of normal lung fibroblasts (WI38) exposed to force showed that the cell bodies elongated and aligned parallel to each other, and parallel to the applied force vectors. This resulted in a strikingly prominent culture morphology, and is in marked contrast to the random distribution of control unstretched Flex II cultures. Alignment is typically complete following four days of mechanical stimulation, under the conditions described above. However, the rate of realignment depends on culture density.

The transformed human lung fibroblasts, rodent cancer or mutant cells failed align in response to applied mechanical force. Culture morphologies in Flex I and Flex II plates were indistinguishable and were characterized by a random distribution pattern of cells.

The unique phenomenon of force-related alignment behavior in normal human lung fibroblasts demonstrates that these cells can both detect and respond to mechanical force. Cells interact with extracellular matrix through transmembrane proteins called integrins, and contact neighboring cells through surface proteins called cadherins. This physical interconnectivity may result in the cellular perception of a force balance resulting from the combination of intrinsic and applied external forces. Integrins act as mechanoreceptors [8], transmitting extracellular forces through integrin/extracellular matrix binding and integrin/cytoskeletal interactions. Transformed, cancer and mutant cells could conceivably have defect(s) in either force perception, integration of force perception, or force response mechanism(s).

Future Directions

This project will be continued during the 1994-1995 academic year at the University of Texas at San Antonio and during the summer (hopefully) of 1995 at NASA-AMES. In particular I want to study (1) the role of cytoskeletal elements such as actin filaments, microtubules and intermediate filaments and (2) the role of integrins and cadherins in the phenomenon of mammalian cell alignment in response to applied mechanical force

References

[1] Vandenburgh, H.H. 1983. Cell shape and growth regulation in skeletal muscle: Exogenous versus endogenous factors. J. Cell. Physiol. 116:363-371.

[2] Vandenburgh, H. 1992. Mechanical forces and their second messengers in stimulating cell growth *in vitro*. Am. J. Physiol. 262:R350-R355.

[3] Komuro, K., Katoh, Y., Kaida, T., Shibazaki, Y., Kurabayashi, E.H., Takaku, F. and Yazaki, Y. 1991. Mechanical loading stimulates cell hypertrophy and specific gene expression in cultured rat cardiac myocytes. J. Biol. Chem. 266:1265-1268.

[4] Brunette, D.M. 1984. Mechanical stretching increases the number of epithelial cells synthesizing DNA in culture. J. Cell Sci. 69:35-45.

[5] Buckley, M.J., Banes, A.J., Levin, L.G., Sumpio, B.E., Sato, M., Jordan, B., Gilbert, J., Link, G.W. and Tay, R.T.S. 1988. Osteoblasts increase their rate of division and align in response to cyclic, mechanical tension *in vitro*. Bone Mineral. 4:225-236.

[6] Buckley, M.J., Banes, A. J. and Jordan, R. 1990. Effects of mechanical strain on osteoblasts *in vitro*. J. Oral. Maxofac. Surg. 48:276-282.

[7] Levesque, M.J. and Nerem, R.M. 1985. The elongation and orientation of cultured endothelial cells in response to shear stress. J. Biomech. Eng. 176:341-347.

[8] Wang, N., Butler, J.P. and Ingber, D.E. 1993. Mechanotransduction across the cell surface and through the cytoskeleton. Science 260:1124-1127.

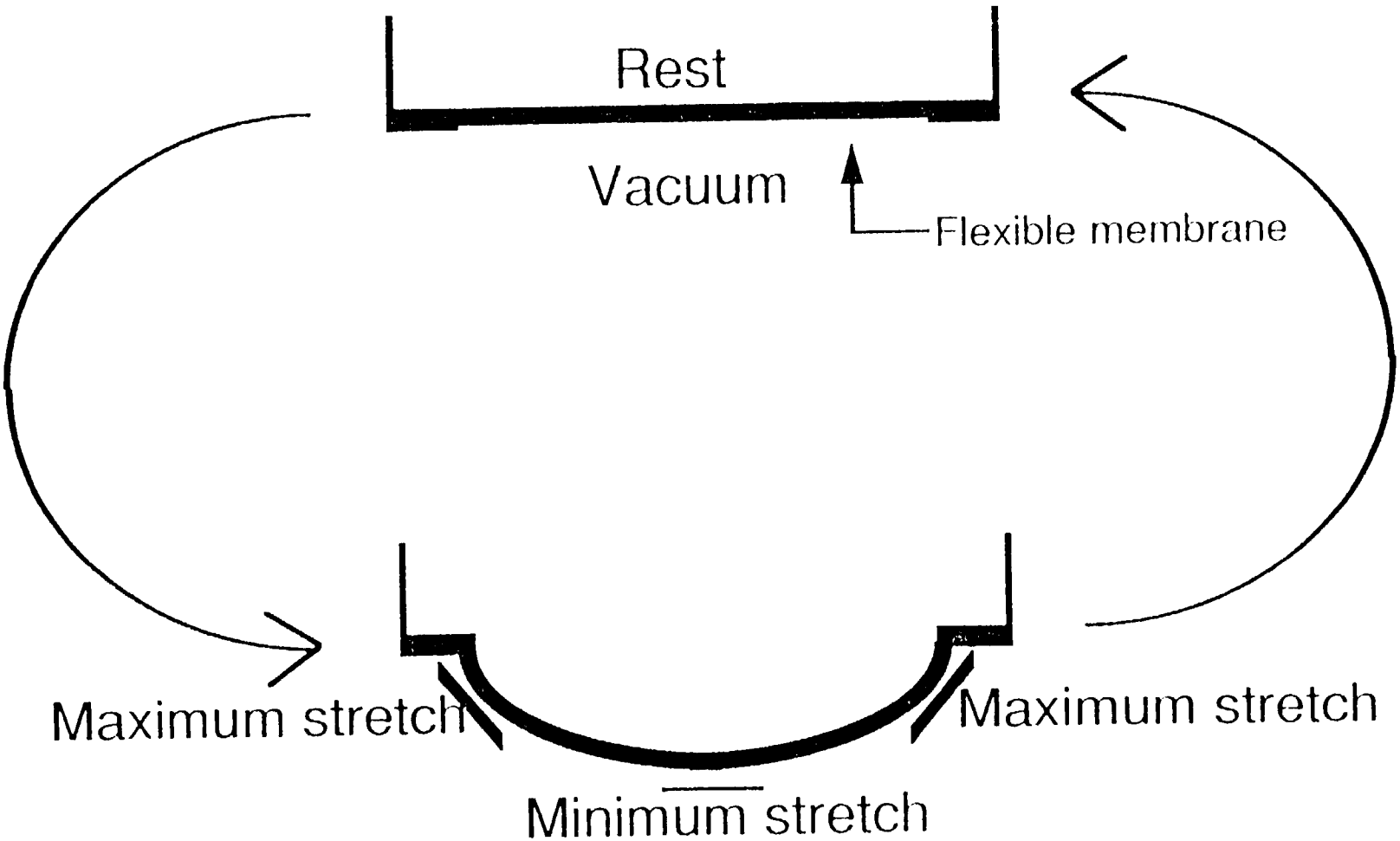

Figure 1. At rest, a well of a 6 well Flex I plate has a flat culture substratum. As negative pressure is applied to the region beneath the silicon elastomer wells, the membrane deforms hemispherically downward, yielding a region of maximum stretch in the outer annulus of the culture well and a region of minimum stretch at the very center of the culture well.